

## Reviewer Report

### Title: Population genomic data reveal genes related to important traits of quail

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#### Reviewer Comments to Author:

In this manuscript, the authors present a chromosome level assembly of the quail genome, and through comparative genomics and RNA-seq address a large variety of questions regarding the relationship of the quail to other galliformes, and identify several important and interesting genes related to various traits such as immunity, sexual maturity and plumage. This is very interesting work, containing well designed experiments and a lot of useful results.

Line 61-65: references required. Or the authors need to clarify that these statements are anecdotal.

Line 78: The sentence "Some of the key avian ... were included[25]." is a poorly formed sentence. I am not sure what the authors are referring to here. I assume that the authors mean that the phylogeny and genetic relationships between the three avian model systems are not well resolved.

Line 118: What do the authors mean by consistency between the previously released genome and their assembly? From Fig S2, it looks like it is synteny, but a little more information on the statistics used to test for consistency between the genomes would be useful.

Line 123-126: The sentence "In addition ... peak-laying (LP) (Table S4)." does not make any sense. It looks like the authors are missing some part of the sentence.

Line 178-181: Number of SNPs identified seems very high implying a very high nucleotide diversity. Were the SNPs identified in the combined populations - so all 31 samples together - or were the SNPs identified using single sample callers. There are caveats in both these methods that could lead to a lot of spurious SNP calls.

Line 181-183: I was a bit confused by what the authors meant by the different number of SNPs in the different populations. Were these the number of SNPs among the ~22 million total SNPs that were polymorphic in each of the three populations?

Line 189-191: The differences in the ratio of non-synonymous to synonymous SNPs in the quail compared to the two other galliforms mentioned might be due to differences in sample size, quality of SNPs, the filtering criteria etc. This might indicate that this ratio is not necessarily significantly different from these other two birds.

Line 195-216: This entire paragraph needs to be rewritten for clarity. It was very difficult to follow the sequence of logical conclusions being laid out by the authors. Firstly, they mention that their estimates of  $\theta_w$  are different in the wild quail compared to the two domestic forms for the autosomes but that their estimate of  $n$  is similar in the three populations. Their conclusion from this is that diversity in the wild quail population is quite high but that the three populations are pretty homogeneous within themselves. This conclusion can be tested by using dimension reduction methods such as PCA, MDS or even AMOVA to see if the within and between group variance support this conclusion. The Z chromosome section talking about higher selective pressure across the entire Z chromosome in the egg type quail is a bit hard to believe and looks like it might be artefactual, since all the genic regions on the Z have similarly low levels of diversity in

all three populations. This implies that the differences on Z have to be driven by non-genic regions. I would need strong evidence to believe that non-genic regions are driving selection across the entire chromosome for this population. An alternative hypothesis for the reduced diversity on the Z might lie in the demography of the egg type. The egg type is also the population with the lowest number of SNPs, indicating that possibly the effective population size of this type is lower than both the wild and meat type, and this in turn means that the  $N_e$  of the Z chromosome is lower in these and since Z is more adversely affected by the reduced  $N_e$  than the autosomes, this effect is still detectable on the Z. Finally, Tajima's D is a statistic based on the differences in  $\theta_w$  and  $\pi$ , I am a bit confused that the autosomes do not show any signal in the D statistic, as the authors previously mention that the  $\theta_w$  for the wild quail is different than that for the egg type and the meat type, while the  $\pi$  are similar. A clarification would be immensely helpful.

Lines 227-229: These sentences talk about the clustering of the wild quail samples on PC1 and PC2. The authors claim that the clustering indicates the homogeneity in wild quail. This is completely false. The first PC is separating out the wild from the domestics and the second PC is separating the egg and meat type. In both these cases, they do not load SNPs that explain the variance within the wild quails. This close clustering of wild quail is to be expected, especially given the low sample sizes in all three groups.

Lines 234-241: Interpretation of the ADMIXTURE results. Firstly, given the admixture plots, it is clear that the admixture proportions being calculated for the 31 samples are not stable for the cases from  $K=4$  to  $K=7$ . The ancestry clusters appear and disappear and clusters found at lower  $K$ s are not found at higher  $K$ s. Interpreting any of these results is inappropriate and does not lead to valid inferences. Therefore, I suggest that the authors restrict their interpretations to  $K=2$  and  $K=3$  which are very nice results. The slight mixing shown between egg and meat type also explains the spread along PC2 in the PCA plot.

Lines 245-248: I am not sure I follow how the quick LD decay is indicative of heterogeneity in populations resulting from gene segregation and high recombination. Firstly, LD computed here is  $r^2$ , which is not an indicator of recombination rates or frequencies, since it depends on the matching of allele frequencies of the two variants under consideration. Second,  $D$  or  $D'$  is a much better candidate for measuring recombination. Third, measure mean pairwise  $r^2$  in windows is highly affected by low frequency variants which by default have low  $r^2$  with other variants. A lower bound on the minor allele frequency would help in this regard. One other thing which might be causing trouble in this and other analyses might be the excess of singletons. An additional column indicating the number of singletons and the proportion of private variants in each of the three populations would be very useful in table 1.

Line 265: "complete bottleneck" is a meaningless term. I think the authors mean to say strong bottleneck.

Lines 283-284: If the authors mean many large scale selection events in the genome, then I agree, but the sentence might need rephrasing. It now sounds like the authors claim multiple selective sweeps in the same loci, which is not possible to tell from the data they use.

Lines 300-301: We do not need to invoke 2 domestications, but can say that the quail was probably domesticated for other traits (as mentioned earlier probably crowing), and the egg and meat traits were selected upon independently from this domesticated from starting from the early 20th century.

Lines 302-304: Did the authors perform RNA seq or similar to measure gene expression? The following paragraph (Lines 305-) does not say anything about expression but about different non-synonymous SNPs in domestics compared to the wild. Although this is very nice analysis, it has nothing to do with differential expression.

Lines 335-340: The authors mention that higher FSH $\beta$  leads to reduction in mature FSH, which is required for sexual maturity. But they also mention that this FSH $\beta$  is higher in quail compared to chicken, which would imply later maturity in quail. I might have missed something but this is counterintuitive.

Lines 411-415: The additional quails added here come from different populations, which have been shown to be different before, so it would be useful to know what kind of corrections were performed for this, since the lower p-value might be an artifact of population structure in the samples.

Language related comments:

Line 27: "That only one of them shared" should read "That only one of them is shared"

Line 29: "marron" should read "maroon" I think.

Line 60: "than that do in males" should read "than males".

I stopped here, but there are lots of English related errors in the manuscript, and in its current form, it cannot be published due to the language issues, although the content deserves the platform.

## **Methods**

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Yes

## **Conclusions**

Are the conclusions adequately supported by the data shown? Yes

## **Reporting Standards**

Does the manuscript adhere to the journal's guidelines on [minimum standards of reporting](#)? Yes

Choose an item.

## **Statistics**

Are you able to assess all statistics in the manuscript, including the appropriateness of statistical tests used? Yes, and I have assessed the statistics in my report.

## **Quality of Written English**

Please indicate the quality of language in the manuscript: Not suitable for publication unless extensively edited

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